Food Chemistry 128 (2011) 543–548

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Geographical origin differentiation of saffron spice (*Crocus sativus* L. stigmas) – Preliminary investigation using chemical and multi-element (H, C, N) stable isotope analysis

Luana Maggi [a], Manuel Carmona [a], Simon D. Kelly [b], Niusa Marigheto [c], Gonzalo L. Alonso [a,*]

[a] Cátedra de Química Agrícola, E.T.S.I. Agrónomos, Universidad de Castilla-La Mancha, Campus Universitario, 02071 Albacete, Spain
[b] Stable Isotope Mass Spectrometry, Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK
[c] Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK

ARTICLE INFO

Article history:
Received 3 March 2010
Received in revised form 9 November 2010
Accepted 11 March 2011
Available online 17 March 2011

Keywords:
Saffron
Geographical origin
Isotopic analysis
Chemical analysis

ABSTRACT

A preliminary study of the bulk hydrogen, carbon and nitrogen stable isotope composition of 28 authentic saffron samples produced from *Crocus sativus* L. cultivated in the typical production areas of Western Macedonia in Greece (8), Khorasan Province in Iran (7), Sardinia in Italy (6) and Castilla-La Mancha in Spain (7) is described. A chemical characterisation of 16 key quality parameters was also completed on the same samples by UV–Vis, HPLC and GC analyses. Multivariate analysis of the data revealed that 60.7% of saffron samples could be correctly assigned to their respective production countries using the chemical parameters. However, the combined bio-element stable isotope data reliably classified 100% of the saffron samples according to their respective geographical origins using posterior cross validation. Further work is required to establish the long-term stability of these models with respect to different years of production and other major producers such as India and Morocco.

1. Introduction

Saffron is one of the oldest and most expensive spices in the world. It is obtained from the red dried stigmas of *Crocus sativus* L. that is cultivated in several countries such as Iran, India, Greece, Morocco, Spain and Italy (Ghorbani, 2006). The price of saffron depends on its quality, which is closely related to the terroir of the production area in an analogous way to wine. Saffron quality is also strictly categorised and controlled according to the ISO 3632 (2003). Its main characteristics are colour (Carmona et al., 2005), taste (Carmona, Sánchez, et al., 2007) and aroma (Carmona, Zalacain, Salinas, & Alonso, 2007). The saffron colour is derived from the presence of various water soluble crocetin esters, commonly known as crocins (Carmona, Zalacain, Sánchez, Novella, & Alonso, 2006), while picrocrocin has always been considered as the main compound responsible for saffron's bitter taste (Corradi & Micheli, 1979) and to a lesser extent kaempferols (Carmona, Sánchez, et al., 2007). Saffron's aroma profile is relatively complex, being derived mainly from safranal (Alonso, Salinas, Estéban-Infantes, & Sánchez-Fernández, 1996).

Previously, Martin, Remaud, and Martin (1995) applied carbon stable isotope analysis to distinguish between safranal extracted from authentic saffron and safranal produced by chemical synthesis. One of the two synthetic safranal samples had a $\delta^{13}C‰$ value close to that of safranal extracted directly from saffron. As these two samples of safranal were indistinguishable, Martin et al. (1995) used this as a justification for the site specific $^{2}H/^{1}H$ ratio analysis of safranal by Nuclear Magnetic Resonance (SNIF-NMR™). Significant differences in the $^{2}H/^{1}H$ ratio were observed at each of the six molecular environments measured by SNIF-NMR. However, only one sample of synthetic safranal and one sample of safranal extracted from authentic saffron were reported and no discussion of the possibility of using isotope analysis to distinguish saffron geographical origin was reported. Semiond et al. (1996) also attempted to distinguish between synthetic safranal and safranal extracted from authentic saffron using carbon stable isotope analysis. They reported the successful discrimination of one synthetic safranal sample and five samples of safranal extracted from saffron using methanol and supercritical fluid extraction. However, the $\delta^{13}C‰$ values of extracted safranal differed significantly from those reported by Martin et al. (1995). Semiond et al. (1996) went onto discuss the use of the measured $\delta^{13}C‰$ values of safranal to distinguish geographical origin of saffron. They concluded that carbon isotope ratio analysis did not provide the means to distinguish the geographical origin of saffron. As *C. sativus* L. uses the Calvin cycle to fix carbon dioxide the major carbon isotope fractionation is that which occurs during photosynthesis irrespective of geographical origin. However, carbon isotope ratios from the same plant species are known to vary globally due to the effects of water stress

* Corresponding author. Tel.: +34 967 599310; fax: +34 967 599238.
E-mail address: Gonzalo.Alonso@uclm.es (G.L. Alonso).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.03.063

on stomatal opening and concomitant effect on carbon dioxide diffusion and fractionation. Colder and more humid climates result in relatively depleted $\delta^{13}C‰$ values compared to $\delta^{13}C‰$ values derived from the same plant species growing in relatively hotter and less humid environments.

Multi-element isotopic analysis has been successfully applied to a range of foodstuffs to develop methods that will permit their geographical origins to be determined (Kelly, Heaton, & Hoogewerff, 2005). Carbon isotopes in foodstuffs do exhibit some geographical dependence linked to water stress and humidity during cultivation but the differences are very small in comparison to oxygen and hydrogen isotopes. The measurement of hydrogen and oxygen stable isotopes are applicable to the characterisation of geographical origin because they depend strongly on the latitude, distance from the sea and altitude, due to fractionation in the global hydrological cycle (Yuntseover & Gat, 1981). It has previously been demonstrated that the 'geographical signal' from water is transferred into plant and animal products (Hobson, 1999). Furthermore, nitrogen isotope compositions provide information about marine and terrestrial plants and also regional agricultural practices, especially the use of fertilisers used in organic and conventional agriculture (Bateman & Kelly, 2007).

Other analytical techniques and parameters have been studied to verify saffron origin such as, in terms of aroma by gas chromatography (Kanakis, Daferera, Tarantilis, & Polissiou, 2004), infrared spectroscopy (Anastasaki et al., 2010; Zalacain et al., 2005) or electronic nose (Carmona, Martínez, et al., 2006), free amino acids (Del Campo et al., 2009) and flavonoids content (Carmona, Sánchez, et al., 2007).

The aim of this research was to apply for first time multi-element stable isotope analysis to distinguish the geographical origin of saffron spice by measuring its hydrogen, carbon and nitrogen stable isotope composition. The isotopic composition was assessed along with chemical composition characteristics such as colour, taste and aroma parameters to establish the extent to which the geographical origin of saffron could be reliably determined using multivariate statistical analysis.

2. Materials and methods

2.1. Samples and reagents

This study involved 28 well-defined samples of saffron from leading producers (8 from Greece, 7 from Iran, 6 from Italy and 7 from Spain) from the same harvesting year (2006). Samples were obtained directly from the producers with the guarantee of their origin and lack of adulteration. Saffron is produced in a relatively restricted number of locations in these countries and so the samples used in this study were typically representative of those that would find their way into retail markets and were derived from *C. sativus* L. cultivated in the typical production zones of Western Macedonia (Greece), Khorasan Province (Iran), Sardinia (Italy) and Castilla-La Mancha (Spain). The samples were kept at 4 °C in absence of light until their analysis.

Cyclohexane, *n*-hexane and formic acid were purchased from Panreac (Barcelona, Spain), while acetonitrile from Scharlau (Barcelona, Spain). Safranal with purity of 98% was supplied by Sigma–Aldrich (Madrid, Spain) while water was purified through a Milli-Q System (Millipore, Bedford, MA, USA).

2.2. Chemical characterisation

2.2.1. Saffron working solution

Five hundred milligrams of ground saffron, previously passed through a sieve of 5 mm pore diameter, was placed in a volumetric flask (1 L) and 900 mL of distilled water were added. The solution was stirred by magnetic bar (1000 rpm) for an hour by keeping it away from light exposure. Subsequently, the flask was then filled to 1 L mark and the solution was homogenised through agitation.

2.2.2. Moisture and volatile matter content

Determination of moisture and volatile matter content of saffron was carried out according to the ISO 3632 (2003).

2.2.3. UV–Vis determinations

The analytical procedure employed to prepare the saffron solution and for recording the absorbance variation followed the specifications established at ISO 3632 (2003), were colouring strength was determined as $E^{1\%}_{1\ cm}$ 440 nm, picrocrocin as $E^{1\%}_{1\ cm}$ 257 nm, and safranal as $E^{1\%}_{1\ cm}$ 330 nm.

2.2.4. Identification and quantification of crocetin esters and picrocrocin by HPLC

Identification of crocetin esters has been carried out according to Carmona, Zalacain, et al. (2006). The nomenclature for the crocetin ester identified was: T-4GG (*trans*-crocetin di-(β-D-gentiobiosyl) ester), T-3Gg (*trans*-crocetin (β-D-glycosyl)-(β-D-gentibiosyl) ester), T-2G (trans-crocetin (β-D-gentiobiosyl) ester), C-4GG (*cis*-crocetin di-(β-D-gentiobiosyl) ester) and C-3Gg (*cis*-crocetin (β-D-glycosyl)-(β-D-gentiobioyl) ester).

The crocetin esters quantification was also estimated using the method based on the extinction coefficient and the related area calculated. Hence, the crocetin ester concentrations were calculated using the following expression: concentration(mg/100 mg) = $(A\times\ 100/A_t) \times (mw/\varepsilon) \times E^{1\%}_{1\ cm}440\ nm/10$, where the extinction coefficient (ε) was 89,000 $M^{-1}\ cm^{-1}$ and 63,350 $M^{-1}\ cm^{-1}$ for *trans*- and *cis*-crocetin esters, respectively (Speranza, Dadà, Manitto, Monti, & Gramatica, 1984). *A* was the area of the crocetin ester peak in the chromatogram and finally A_t was the total area of the crocetin esters. Finally $E^{1\%}_{1\ cm}440$ nm was the colouring strength of the samples, and mw was the molecular weight of the crocetin ester identified and quantified: T-4GG 977 g mol^{-1}, T-3Gg 815 g mol^{-1}, T-2G 653 g mol^{-1}, C-4GG 977 g mol^{-1}, C-3Gg 815 g mol^{-1}.

The identification of picrocrocin in the samples was carried out by the standard isolated according to Sánchez, Carmona, del Campo, and Alonso (2009). Picrocrocin calibration curve was: picrocrocin amount (mg picrocrocin/100 mg saffron) = (0.0708 ± 0.0004) × 50 × Area/m, R^2 = 0.9998, where Area was the area of the peak of picrocrocin in the chromatogram at 250 nm, *m* was the mass of the saffron and 0.0708 was the slope and 50 was the volume of the sample multiplied by 100 to the units correction.

2.2.5. Identification and quantification of volatile compounds by TD–GC–MS

The determination of volatile compounds was carried out according to Maggi et al. (2009). Identification was carried out by the selection of their respective *m*/*z*, using the NIST library. Not all volatile compounds present in saffron are commercially available as standards, so safranal has been used as external standard. The quantification of volatile compounds was performed with the equation (mg safranal kg^{-1} saffron = 10.88 + 36.76 × $Area_{safranal}$ where $Area_{safranal}$ = safranal peak area/10^6 in the GC chromatogram; R^2 = 0.998).For each sample, every chemical determination was carried out by triplicate.

2.3. Isotopic characterisation

2.3.1. Extraction procedure for isotopic analysis

Two hundred and fifty milligrams of ground saffron were weighed and introduced into a centrifuge tube and 10 mL of